

The reactions were incubated for 1 h at 37 °C and terminated by heating to 70°C for 10 min.

The *ob* cDNA product was amplified by PCR using the following degenerate primers with restriction site linkers for BamHI and XbaI respectively; sense strand 5'-

GTGCCYATCCARAAAGTCC-3' (SEQ ID NO: 9) and antisense strand 5'-

GCAYYCAGGGCTRASRTC-3' (SEQ ID NO: 10) Adipose tissue cDNA was added as

template to 50 µl PCR reactions made in the manufacturer's buffer with 100 pmol of each

primer and 2.5 U of Taq DNA polymerase (LifeTechnologies, Inc.). A three stage

amplification was carried out under the following conditions; Stage 1- 95°C, 3 min; 52°C, 1

min; 72°C, 1 min; 1 cycle; Stage 2- 94°C, 45s; 52°C, 45s; 72°C, 1 min; 4 cycles; Stage 3-

94°C, 45 s; 55°C, 30 s; 72°C 1 min; 28 cycles. The PCR products were digested with the

restriction enzymes BamHI and Xba I and purified by electrophoresis in 1% NuSieve low

melting point agarose (FMC Bioproducts, Rockland, ME, USA). The *ob* cDNA was ligated

into Bluescript II SK+ (Stratagene Inc. LaJolla, CA, USA) and transformed into MCR DH5α

(LifeTechnologies, Inc.) and plated on LB plates containing 50 µg/ml ampicillin for plasmid

selection. Twelve *E. coli* colonies were isolated that contained the porcine *ob* cDNA and

plasmid DNA was isolated for sequencing. Dideoxy sequencing reactions were carried out

using [³⁵S] dATP labeling with Sequenase V2.0. The sequence samples were loaded on

5% Long Ranger (FMC Bioproducts) for denaturing gel electrophoresis according to the

manufacturer's recommendations.

Please delete the paragraph on page 32, lines 7-19, and replace it with the following paragraph:

Rabbit polyclonal antibodies were initially made to synthetic peptides derived from the N-terminus of the secreted portion of the protein (WRVQDDTKTLIKTIVTRISD) (SEQ ID NO: 11) as a map peptide and the C-terminus (C1 peptide, LQGALQDMLRQLDLSPGC) (SEQ ID NO: 12) for conjugation to keyhole limpet hemocyanin. Both peptides produced antibodies in rabbits that cross-react with the recombinant pig leptin produced using the